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B. Webb
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PATENT
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In re Application of :	Kirk Hogan	
Serial No.:	09/613,887	Group No.: 1655
Filed:	07/11/01	Examiner: J.E. Goldberg
Entitled:	Methods and Compositions for Perioperative Genomic Profiling	

DECLARATION OF KIRK HOGAN, M.D. UNDER 37 CFR §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being sent by facsimile transmission to the U.S. Patent and Trademark Office, via Examiner J.E. Goldberg at (703) 746-5149.

Dated: 2-8-02 By: Mary Ellen Waite
(Mary Ellen Waite)

Madam:

1. I, Kirk Hogan, am the inventor of the subject matter embodied in the above-identified patent application.

2. I am a licensed and board-certified anesthesiologist, and am an Associate Professor in the Department of Anesthesiology at the University of Wisconsin Medical School.

3. As an instructor and licensed and board-certified practitioner, I am knowledgeable about the practice of anesthesiology.

4. Patients undergoing surgery and anesthesia exhibit wide variation in their physiologic and pathologic responses to drugs and trauma, such that an intervention that would be perfectly safe in one individual carries the potential for grievous harm and even death to another.

5. A substantial proportion of inter-individual variation is genetic, but, to my knowledge, prior to the present invention, this risk was unaccounted for in clinical practice by any technology, or by routine, direct molecular genetic analysis.

6. At present, to determine genetic risks before surgery, doctors ask if the patient has ever had a problem with anesthesia or surgery, and whether any of their family members have had a problem. This is the extent of the standard perioperative genetic evaluation unless an earlier record of perioperative care is available, in which case it is reviewed.

7. I am not aware of any case where physicians have carried out genomic profiling in the perioperative period using a heterogenous assay (other than my own work).

8. This is not surprising because the state of the art in the surgical and anesthetic arts at the time the application was filed teaches that perioperative genetic testing should not be conducted.

9. Even to this day, to my knowledge, perioperative genetic testing is not carried out.

10. In practice today, perioperative testing (even for biochemical testing) of healthy individuals is minimized or avoided. This is evidenced, for example, in a number of modern texts, manuals, and articles. For example, the 2002 Pediatric Anesthesia text by Gregory (attached) states that routine perioperative testing (biochemical tests) in healthy infants and children should be avoided. The 2002 Clinical Anesthesia Practice text by Kirby et al. (attached) states that routine testing for healthy individuals is unnecessary.

11. I filed a grant application entitled "Perioperative Genomic Profiles" with the Anesthesia Patient Safety Foundation (APSF), an organization dedicated to safety in the perioperative interval. The grant application described the subject matter of the present invention and was rejected. The review committee, consisting of experts in the field concerned with safety in the perioperative period, on 11/27/2000, explained that the invention goes in the opposite direction from the state of the art. The committee's comments, in full, are provided below:

> "The APSF committee members reviewing your proposal to study genomic profiles were impressed by the elegance of the proposal. It would take the issue

of patient safety in a new direction. It could improve the safety of the anesthetic experience, particularly for those patients with unknown diseases.

The committee's concern and reason for not funding the study rested on a few factors. It is a basic science study without clear clinical value. In the value equation the committee members considered the study might improve quality but the cost could be very high. As anesthesia practice has moved toward determining the ratio of quality to cost, this study seems to be going in the opposite direction. It suggests we screen everyone in the hopes we find something on almost everyone. The direction of anesthetic evaluation is presently to *not* routinely do any preoperative studies.

The committee members were also concerned that patient confidentiality and ethics are problematic. Do patients want to know all that is potentially wrong with them? The committee members were concerned that the findings of the study could well increase costs with little benefit to the patient."

12. The review committees' comments demonstrate that experts in the field of perioperative medicine do not believe that perioperative genetic testing should be carried out—even after reading my grant proposal. It is clear to me, based on my knowledge and experience in the field, that they would also not believe perioperative genetic testing should be carried out in view of the references cited in the present Office Action (which they have reviewed by references in the grant application itself)—references that do not teach that perioperative testing should be carried out, and that do not provide guidelines for selecting markers useful for perioperative genetic testing.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: 2/7/02 Signed: _____
Kirk Hogan

Pediatric Anesthesia

Fourth Edition

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2002

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current status of the child's asthma. Children with clinical evidence of significant bronchospasm should not be accepted for elective surgery, and it is preferable to defer surgery on those who have had an acute asthma attack within the past 4 weeks. A history of a previous ICU admission or systemic corticosteroid therapy should be sought. Supplemental steroid therapy should be ordered for those who have had recent or prolonged corticosteroid therapy. Patients receiving theophylline therapy should have a blood level determined preoperatively; elevated theophylline levels may cause serious intraoperative arrhythmias. Asthmatic children can usually be effectively prepared for elective surgery by optimizing their maintenance therapy and adding preoperative inhalations of β_2-adrenergic agonist agents (e.g., albuterol) as necessary. The anesthesia technique should be carefully designed to include bronchodilator agents (e.g., sevoflurane) and to avoid histamine-releasing drugs (e.g., *d*-tubocurarine, atracurium, or morphine).[35]

ROUTINE PREOPERATIVE LABORATORY STUDIES

In recent years, the value of routine preoperative screening tests for healthy infants and children has been questioned, and consequently less testing is now performed. Routine chest radiographs have never been considered a part of the evaluation process of the healthy child. Routine urinalysis has not been found useful in detecting important diseases in a population of healthy pediatric surgical patients,[36] and most hospitals and surgical centers now omit this test. Routine preoperative hemoglobin or hematocrit determinations have been recommended in the past, and have been or still are required by law in some jurisdictions. However, there are few data to support the practice of subjecting every healthy child to a painful fingerprick or venipuncture. Recent studies[36, 37] indicate that the incidence of serious anemia is very low in "healthy" pediatric surgical patients. Mild degrees of anemia do not usually affect the decision to proceed with the operation or result in any modification of the anesthesia management.[37, 38] Significant anemia, when present, is usually found in young infants or in older children with chronic diseases. There is a growing movement to omit all routine testing and to test only those patients at risk for more severe and physiologically important anemia. These would include all infants under the age of 1 year, patients with a chronic disease, all those at risk of having a hemoglobinopathy (e.g., sickle cell disease), those with symptoms or signs of anemia, and those who "just don't look well." In addition, patients with the potential for significant intraoperative blood loss should undergo baseline hematologic studies.

The present consensus, therefore, is that routine screening tests are of little value. When these are omitted, however, the place of a careful and thorough preoperative assessment, considering all aspects of the child's health, assumes great importance.[38]

The child who is found to be anemic should be carefully assessed but, as stated above, mild degrees of anemia are usually not contraindications to necessary minor surgery. There is no justification for preoperative transfusion unless the anemia is severe and symptomatic, or significant cardiorespiratory disease is present. Unexpected anemia should be investigated to determine its cause, and suitable medical therapy can then be applied. In some cases it may be appropriate to defer purely elective surgery until the medical treatment of anemia is completed. In cases of nutritional anemia, oral iron therapy can be expected to increase the hemoglobin level within a few weeks.

PREOPERATIVE FASTING: WHAT IS NECESSARY?

It is now well recognized that withholding fluids preoperatively is distressing to a child and may result in significant fluid depletion. In the past, children have often suffered through many hours of fluid restriction. Over the past few years, a much more liberal approach to the matter of preoperative fasting has emerged. This is based on a number of good studies that clearly demonstrate that prolonged periods of fluid deprivation are unnecessary.[39] The volume and acidity of the stomach contents of healthy children are not increased in those who have reasonable amounts of clear fluid 2 hours preoperatively compared with those who are fasted for 6 to 8 hours.[40] Therefore, most authorities now favor allowing reasonable amounts (3 ml/kg) of clear fluids orally (PO) until 2 hours before induction of anesthesia.[41]

Solid and semisolid foods are of more concern, as they may remain in the stomach for longer periods. In general, it is common practice to omit food on the day of surgery for morning cases and to allow only a small, soft breakfast for afternoon cases.

Infants who are being breast-fed should complete their last preoperative feeding at a time preoperatively that is equal to the usual interval between feedings. Thus, if the infant is being fed every 4 hours, the last feeding should be completed 4 hours before operation. Such infants may be offered clear fluids, if they will take them, until 2 hours preoperatively.

Children who are not otherwise healthy and those who present for emergency surgery require special consideration. Those with gastrointestinal disease or history of gastroesophageal reflux are at special risk for regurgitation during induction of anesthesia. The volume and acidity of the stomach contents of children who present for emergency surgery may constitute a significant hazard. Gastric emptying is delayed after trauma; even 6 to 8 hours later, large volumes of acid contents may be present.[42] The volume and acidity of gastric contents are similar in children with orthopedic or urologic disease to those with abdominal emergencies.[43] Children about to undergo emergency surgery should receive no further fluids PO and may benefit from intravenous fluids and therapy to reduce the volume and acidity of the gastric contents. Glycopyrrolate (0.1 mg/kg intramuscularly [IM]) reduces the volume and acidity of the stomach contents of children.[44] Cimetidine (5 mg/kg PO) reduces gastric acidity, and when given to children by the rectal route in a dose of 40 mg/kg also reduces the volume of gastric contents.[45] Cimetidine also may be infused intravenously (IV) over 15 to 30 minutes in a dose of 5 to 10 mg/kg. Metoclopramide 0.15 mg/kg

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Clinical Anesthesia Practice

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TABLE 1–4. Useful Bedside Maneuvers to Determine the Type of Cardiac Murmur

Type of Murmur	Müller Maneuver	Valsalva Maneuver	Squatting	Standing	Amyl Nitrate
Right-sided heart murmurs	↑		Should ↑		
Hypertrophic cardiomyopathy	↕	↑	↓	↑	↑
Aortic stenosis		↕	Should ↑	↓	↑
Mitral regurgitation			Should ↑	↓	↓
Mitral valve prolapse		±		↓	±
Aortic insufficiency			↑		↓
Pulmonic stenosis				↓	↑
Tricuspid regurgitation				↓	↑
Pulmonic regurgitation					↑

Reproduced with permission from Rothman A, Goldberger AL. Aids to cardiac auscultation. *Ann Intern Med.* 1983;99:346.

Heart

Examination of the heart should include an assessment of the heart rate and rhythm (regular, irregular, presence of extrasystolic beats) and determination of whether murmurs, extra heart sounds (eg, a third heart sound), or jugular venous distention are present. Determination of the type of murmur may be assisted by the use of amyl nitrate and several bedside maneuvers (Table 1–4).[100, 101]

Extremities and Back

The extremities and back should also be examined. If use of a regional anesthetic is contemplated, examination of the injection site is important to identify that it is clear of lesions and that the appropriate landmarks are present to determine the feasibility of the technique.

A modification of the Allen test (Table 1–5)[102] is usually performed to provide some assessment of the adequacy of ulnar collateral flow if cannulation of the radial artery is considered. However, the utility of this test in patients without PVD has been called into question.[103] Because this test does not cost anything and has no risk associated with it, and because the results may alter choice of the cannulation site, it is difficult to convincingly argue against its use.

Neurologic Function

If a regional anesthetic technique is planned, the anesthesiologist should document the neurologic function in the area to be anesthetized. Likewise, if the patient is to be operated on in an unusual position, the anesthesiologist should determine the neurologic function of areas that could be affected by the position. A corollary to this approach is to have the patient assume the anticipated intraoperative position to determine whether it is easily tolerated.

TABLE 1–5. Modified Allen Test

Both radial and ulnar arteries are compressed
The patient clenches and unclenches the fist repeatedly until the palm develops pallor
One of the arteries is released
The amount of time required for blushing of the palm is noted
The procedure is repeated for the other artery
Normal palmar blushing should be evident within 7 s
An equivocal test is 8-14 s
An abnormal test is present if it takes 15 s or longer for the palm to blush
A pulse oximeter may also be helpful to determine the time to return of flow

From Seneff M. Arterial line placement and care. In: Irwin RS, Cerra FB, Rippe JM, eds. *Intensive Care Medicine.* 4th ed. Philadelphia, Pa: Lippincott-Raven; 1999:36–46.

Preexisting Deficits

Documentation of preexisting neurologic deficits is important, especially considering that 15% of closed malpractice claims made against anesthesiologists involved peripheral nerve injury after anesthesia.[104] If a neurologic deficit is present, succinylcholine may be contraindicated owing to increased muscle membrane chemosensitivity and resultant hyperkalemia.[38]

LABORATORY TESTING

What Tests Are Appropriate?

Extensive information has been published since the 1990s regarding routine preoperative blood tests. The most important screening "test" to detect disease processes is still a thorough history and physical examination.

Routine Testing

There are abundant data supporting the concept that routine laboratory screening tests are not cost-effective in the asymptomatic patient.[1] Tests are often inefficient and do not always identify symptomatic disease.[105] Because a *normal* laboratory test result is usually defined as the mean value for the test ± 2 SD, an *abnormal* test result predictably appears in 5% of the healthy population[106] and increases with the number of tests performed. The probability of healthy individuals having a completely normal 12-test biochemical profile is only 54%.[106]

In addition to the inefficiency of routine testing, abnormalities that are discovered frequently do not have a measurable effect on perioperative anesthetic management or on patient outcome; furthermore, an abnormal test result may cause the ordering of other tests, which increases risk to the patient.[1,107]

For the apparently healthy, asymptomatic male who is <40 years of age and who is undergoing surgery with minimal expected blood loss, no preoperative blood testing is necessary.[1,107] Those patients with underlying disease as detected by history and physical examination should undergo preoperative testing.[1,107,108]

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Malignant hyperthermia: advances in clinical management and diagnosis

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Malignant hyperthermia (MH) was the name given to a type of severe reaction under general anaesthesia that was first described in 1960.[21] Monitoring during anaesthesia at that time was based on clinical observation and physical signs, without the luxury of today's advanced equipment. The apparent features of the reactions were, therefore, dominated by a progressive pyrexia that usually led to death,[11 19 21 22] hence the name malignant hyperpyrexia or hyperthermia. The 'malignant' part of the name MH has proved useful in emphasizing the potentially fatal nature of the reaction. The 'hyperthermia' component is, perhaps, less useful because it identifies a relatively late feature of a reaction in which early intervention is important. From this point of view, it might be useful for the anaesthetist to think of MH as 'malignant hypermetabolism', because the earliest clinical features (rising end-tidal carbon dioxide concentration and tachycardia) are indicative of the metabolic nature of the reaction.

In 1988, a postgraduate educational issue of the *British Journal of Anaesthesia*[33] was devoted to a series of review articles on the subject of MH. Since then, MH research has benefited from the rapid advances in molecular genetics stemming from development of the polymerase chain reaction.[101] Elucidation of the molecular genetic basis of MH carries the prospect of presymptomatic diagnosis and a complete understanding of the aetiology of the disorder and is likely to contribute to our understanding of skeletal muscle pathology and molecular mechanisms of inhalational anaesthetics. These prospects have, however, been made less tangible by the apparent complexity of the molecular genetics of MH.[65 111] Contrary to an earlier view that DNA-based screening could be applied readily,[57] the complexity of the disorder has required a re-examination of the criteria for the clinical diagnosis of MH, refinement of *in vitro* muscle contracture tests (IVCTs) used for laboratory diagnosis and functional analysis of potential subcellular targets of the MH defect(s).[65] In this review, I will focus on these aspects of MH, along with developments in the clinical management of MH since 1988. I will not discuss the management of an MH reaction following its diagnosis, as this is essentially unchanged from earlier reviews.[33]

Clinical aspects of MH

Clinical presentation

Preoperative diagnosis

MH is a manifestation of exposure of susceptible individuals to triggering drugs. Without such exposure, it is usually impossible to identify the MH-susceptible patient unless there is a personal or family history suggestive of MH. Even then, further questioning about the details of the history will often lead to the exclusion of MH. Various musculoskeletal abnormalities, such as scoliosis, hernias or strabismus, have been associated with MH susceptibility,[10 115] but an analysis of >2500 patients tested for MH susceptibility could find no evidence to support such associations.[52] In 1988, Brownell concluded that, of the muscle diseases associated with MH, only central core disease appeared to be truly linked.[13] More recent studies have indicated that not even this link is consistent.[20 54 71]

Some syndromes with features similar to those of MH have been investigated for common aetiology. Neuroleptic malignant syndrome is discussed in the accompanying review by Adnet;[5] these patients need not be considered at risk of developing MH under anaesthesia. There is less certainty concerning those with a history of exertional heat stroke or exercise-induced rhabdomyolysis. Our group and others have demonstrated muscle abnormalities similar to those found in MH in some patients with such a history.[50 62] In our cases, the same muscle abnormality was found in relatives, but was probably not identical to that found in MH.[62] There are undoubtedly 'non-muscle' causes of heat stroke, such as obesity, concurrent viral illness or recent alcohol consumption,[25] but if these are excluded, I would advise that a patient with a history of exertional heat stroke or exercise-induced rhabdomyolysis, especially more than one episode, be investigated for MH.

Table 1 Causes of increased serum creatine kinase concentration. These can be divided into 'identifiable' and 'idiopathic' according to how easy the underlying cause is to diagnose. Hypothyroidism is by far the commonest cause, followed by myopathies

'Identifiable'	Hypothyroidism, myopathies, exercise, malignancy (always BB isoenzyme), denervation syndromes, myocardial infarction, rhabdomyolysis (trauma, drugs)
'Idiopathic'	Malignant hyperthermia susceptibility, Duchenne dystrophy carriers, idiopathic paroxysmal rhabdomyolysis, haemolytic syndromes, previous neuroleptic malignant syndrome

Table 2 Clinical features of malignant hyperthermia. The changes are grouped according to their timing of onset in the reaction. The time periods (early, succeeding and late) cannot be quantified because they vary greatly between patients; they do indicate a consistent temporal relationship in the onset of clinical features. Features in brackets may have their onset at any stage of the reaction. SV=spontaneous ventilation

Timing	Clinical signs	Changes in monitored variables	Biochemical changes
Early	Sustained jaw rigidity after succinylcholine		
	Tachypnoea (SV)	Increased minute ventilation (SV)	
	Rapid exhaustion of soda lime	Rising end-tidal carbon dioxide	Increased Pa_{CO_2}
	Hot soda lime canister		
	High pulse rate	Tachycardia	Decreased pH
	(Irregular pulse)	(Ventricular ectopics)	(Increased $[K^+]$)
		(Peaked T waves on ECG)	
Succeeding	Patient hot to touch	Rising core body temperature	
	Cyanosis	Falling Sp_{O_2}	Decreased Pa_{O_2}
	Dark blood in wound		
	(Irregular pulse)	(Ventricular ectopics)	(Increased $[K^+]$)
		(Peaked T waves on ECG)	
Late	Generalized muscle rigidity		
	Prolonged bleeding		
	Dark urine		Increased creatine kinase, myoglobinuria
	Oliguria		
	(Irregular pulse)	(Ventricular ectopics)	
		(Peaked T waves on ECG)	(Increased $[K^+]$)
	Death		

Table 3 Differential diagnoses of a suspected malignant hyperthermia reaction

Inadequate anaesthesia or analgesia
Inappropriate breathing circuit, fresh gas flow or ventilation
Infection or sepsis
Tourniquet ischaemia
Anaphylaxis
Phaeochromocytoma
Thyroid storm
Cerebral ischaemia
Other muscle diseases

Occasionally, a 'routine' preoperative biochemical screen will reveal an increased raised serum creatine kinase concentration in an apparently asymptomatic patient. This could be indicative of MH, but investigation for MH need be done only if more common causes of creatine kinase elevation and uncommon causes requiring less invasive diagnostic techniques than MH (Table 1) are excluded.

Presentation during anaesthesia

There is no clinical feature that is specific for MH. Diagnosis depends on a knowledge of features that can occur during an MH reaction (Table 2), recognition of these features occurring in a pattern (including temporal relationships between individual manifestations) consistent with an evolving MH reaction, and exclusion of other causes of these clinical features (Table 3). Early diagnosis is important, as prompt, appropriate treatment is associated with the best outcome.

(i) Masseter spasm. In the absence of a family history of MH, the first indication that an individual may be susceptible to the condition is the development of an exaggerated initial response to succinylcholine, i.e. an increase in tension of the jaw muscles.[17] If sufficiently sensitive measuring equipment is used, jaw stiffness after administration of succinylcholine can be detected in most individuals.[81] It is often more pronounced in children.[123] When the jaw stiffness is severe, and especially when it is prolonged, the condition is called masseter spasm. Doubt has been expressed as to the validity of masseter spasm as an indicator of potential MH susceptibility,[86 122] but this doubt was based on a false premise.[33 60] Interestingly, several of the patients included in the report by Littleford and colleagues[86] have been proven subsequently to be susceptible to MH. A further illustration of the need to consider a patient developing masseter spasm following succinylcholine as potentially susceptible to MH is presented by Ramirez and colleagues.[109] These workers reported the case of a trauma victim in whom tracheal intubation in the

accident and emergency department was difficult because of increased resistance to mouth opening following the use of succinylcholine. Anaesthesia was maintained using an infusion of propofol during transfer to the CT scanner and from there to the operating theatre. Once in the operating theatre, for fixation of a humeral fracture, isoflurane was substituted for propofol and subsequently the typical metabolic features of MH developed.[109]

(ii) Hypermetabolism. MH occurs because the triggering drugs cause an imbalance in Ca^{2+} homeostasis within skeletal muscle cells.[87 113] The increased intracellular Ca^{2+} concentration stimulates metabolism both directly, through activation of phosphorylase to increase glycolysis, and indirectly, because of an increased demand for ATP production. ATPases are important components of myofilament relaxation and the Ca^{2+} sequestration pumps of the sarcoplasmic reticulum and sarcolemma. Metabolic stimulation leads to increased carbon dioxide production (tachypnoea and increased end-tidal carbon dioxide concentration) and early lactic acidosis (possibly related to acute intracellular inorganic phosphate deficiency). The resulting mixed respiratory and metabolic acidosis stimulates sympathetic outflow, leading to tachycardia. In humans, changes in arterial pressure are not usually marked in the early phases of MH; this may reflect opposing effects of increased sympathetic drive and locally mediated peripheral vasodilation secondary to tissue acidosis. Increasing body temperature is a relatively late indicator of the hypermetabolic response.[109]

(iii) Muscle rigidity and breakdown. Generalized muscle rigidity can occur in association with masseter spasm following succinylcholine and usually indicates MH susceptibility.[31] As with masseter spasm, it resolves spontaneously, usually within 5 min. A more insidious development of generalized skeletal muscle rigidity during maintenance of anaesthesia with potent inhalational anaesthetics is a sinister feature of MH. It indicates that the ability of the muscle to produce ATP, a function crucial to the reversal of the MH process even if Ca^{2+} release can be halted, is almost exhausted. Sustained rigor also restricts the circulation to the muscle beds, resulting potentially in accumulation of toxic metabolic products and heat, and failure of delivery of oxygen and therapeutic agents to the muscle.

Evidence of rhabdomyolysis can usually be obtained by measuring serum creatine kinase and urinary (or serum) myoglobin concentrations. The time-course of changes in the serum concentrations of these proteins has been described.[80] The magnitude of these changes following MH reactions is, however, quite variable. In some cases, the changes may be indistinguishable from those resulting from surgery,[79] but in others, especially when succinylcholine and a potent inhalational anaesthetic have been used in combination, the creatine kinase concentration may be a thousand times normal. Rhabdomyolysis also leads to extrusion of K^+ from the damaged muscle. Hyperkalaemia and sympathetic stimulation are the main causes of cardiac arrhythmias in MH.

Postoperative presentations

The onset of MH varies in its speed. In some cases, metabolic stimulation will be evident clinically within 10 min of the administration of a potent inhalational anaesthetic; in others, several hours may elapse. It is plausible that the speed of onset reflects the rate of increase in intracellular Ca^{2+} concentration, which will depend on the particular drug used, the concentration of the drug in the muscle and any number of physiological variables that dictate the efficiency of Ca^{2+} homeostatic processes in the individual. Considered together with the complete range of duration of surgical procedures, this variability in timing of onset and rate of development of MH dictates that a procedure may conclude just before the symptoms of MH become apparent. In this situation, the reaction will progress while trigger drug concentrations in the muscle remain above a threshold value. It is possible that lower concentrations of trigger drugs are needed to maintain a reaction than to initiate it because an increased intracellular Ca^{2+} concentration stimulates sarcoplasmic reticulum Ca^{2+} release through the mechanism known as Ca^{2+}-induced Ca^{2+} release.[75 129] Similarly, if very high intracellular Ca^{2+} concentrations are reached, Ca^{2+}-induced Ca^{2+} release may sustain an MH reaction even when the trigger drug has been eliminated, especially if Ca^{2+} sequestration is compromised by a reduced capability for ATP production. This is probably why recrudescence of signs can occur after their initial control.

MH reactions that begin soon before or after the end of surgery do, however, present with the same features as intraoperative reactions. This is important because it means that postoperative pyrexia, in the documented absence of tachypnoea, raised end-tidal partial pressure of carbon dioxide and tachycardia, is *not* indicative of MH.[53] It is important to stress that MH cannot be excluded as the cause of postoperative pyrexia if records of anaesthesia and the immediate postoperative period do not detail variables indicative of hypermetabolism. Occasionally, no metabolic features of an MH reaction are recognized during the perioperative period (they may, for example, be attributed to sepsis) and the patient will present 2–4 days after the operation in acute renal failure secondary to rhabdomyolysis.[12]

Clinical diagnosis

None of the clinical features of MH is specific to MH (Table 2) and a variety of differential diagnoses exists (Table 3). Several attempts have been made to analyse clinical features of MH reactions for their use in diagnosis.[31 49 76 78] Each of these studies used a different approach and three will be discussed further. Ellis and colleagues,[31] based on a suggestion by Ording and Ranklev (see ref. 52), defined

eight categories of clinical presentation. Three of these included reactions that were predominantly metabolic, but : different severity; a further three categories were for masseter spasm, either alone, with other 'muscle' features (high creatine kinase, generalized muscle rigidity) or with metabolic signs; the other categories were unexplained anaesthetic death or cardiac arrest and 'other presentations', e.g. postoperative pyrexia or renal failure.[31] Four hundred and two index patients were assigned to these categories; for each category, the proportion of patients diagnosed as susceptible to MH using IVCTs was calculated. This approach has proved useful in providing a rough estimate of the likelihood of MH (and the need for definitive testing) in patients who have experienced an adverse reaction which was thought to be MH. Further use of these data is limited because they were inevitably dependent on contemporary anaesthetic practice and the referral pattern to our unit. For example, I suspect that patients with lesser degrees of masseter spasm are referred now, compared with 15–20 years ago, because of the prevalence of articles on this subject in the intervening period.

Hackl and colleagues[49] also compared results of IVCTs with clinical signs in index cases, but with a more sophisticated statistical approach. They generated logistic regression models in an attempt to predict MH susceptibility. However, the best model could correctly classify only 78% of subjects, highlighting the difficulty in predicting MH susceptibility from clinical features.

Rather than analysing data from a sample of patients, Larach and colleagues used the Delphi process to generate iteratively a consensus clinical grading scale for MH.[78] Eleven experts responded to a series of questionnaires in which they were asked to score the relative importance of potential clinical indicators of MH, with feedback and anonymous comments from the other members of the expert panel. Scoring was ultimately used to generate a qualitative indication of likelihood of MH susceptibility.[78] This study has proved invaluable in validating the laboratory diagnostic techniques for MH susceptibility[7 103] because 'true' diagnosis of MH susceptibility obtained using the 'almost certain' descriptor of the clinical grading scale[78] was derived totally independently of contracture test results. However, the clinical grading scale can be criticized. First, no weighting is given to the temporal relationship of events, which can be used to exclude MH in some situations. Second, the score will be influenced greatly by missing data. Most importantly, the scores and rules for their application are somewhat ambiguous, leading to considerable variability in scoring, even among the 11 experts used to generate the clinical grading scale.[78]

Pharmacological triggers of malignant hyperthermia

: potent inhalational anaesthetic drugs are the most commonly implicated pharmacological triggers of MH. Indeed, there are no verified cases of death from MH where a potent inhalational anaesthetic has not been used. There remains uncertainty over some groups of drugs because of the potential limitation in extrapolating *in vitro* data to exclude a drug, that has been implicated on the basis of a clinical report, as a trigger. Over the past 30 years, many groups of drug have been implicated when one of their number was used as part of a combination of drugs providing anaesthesia for patients who subsequently developed an MH reaction during surgery. The situation is complicated further by a lack of verification that the clinical reaction was a true MH response rather than being caused by other factors, such as sepsis, inadequate anaesthesia or analgesia or, more rarely, thyrotoxicosis or phaeochromocytoma.

Potent inhalational anaesthetic drugs

Although MH was not recognized until the introduction of halothane, it is likely that the classical anaesthetic vapours, diethyl ether and chloroform, in addition to other now unused ether derivatives, accounted for many fatal cases of MH.[22 55] *In vitro* studies have demonstrated that ether and chloroform cause contracture in MH muscle (unpublished observations) and it is probable that these early MH reactions went unnoticed, because of the lack of routinely available monitoring systems and, perhaps, because death under anaesthesia was relatively common. Of the contemporary potent inhalational drugs, halothane is the most potent at inducing sustained contraction in isolated muscle strips from MH patients. The frequency of implication of the individual volatile drugs as triggers in the clinical setting reflects, however, the frequency of their use in anaesthesia, with isoflurane being the most commonly implicated trigger in the UK at present. Sevoflurane, which has only recently been introduced into European and North American practice, has been used for several years in Japan and has triggered MH reactions in several reported cases.[27 105] The other recently introduced drug, desflurane, has also been reported to cause MH.[6 41 96]

In vitro challenge of skeletal muscle with halothane has formed the basis of diagnostic testing for MH for nearly 30 years.[28 30] Similar *in vitro* challenge tests have been used with other drugs to test their potential for triggering human MH.[39] When conducting such tests, it is vital that appropriate concentration ranges of the test drug are used. This is relatively easy with potent inhalational drugs because the partial pressure of the agent used to produce clinical anaesthesia is easily extrapolated and this can be reproduced in the laboratory. With other drugs, account must be taken of the volume of distribution and, especially, the plasma protein binding when calculating the active clinical concentration by extrapolation from the clinical dose range.

Neuromuscular blocking drugs

As discussed above, masseter spasm after the use of succinylcholine is often the first indication that a patient may be susceptible to MH. *In vitro* experiments demonstrate that succinylcholine will cause an increase in intracellular

Ca^{2+} concentration in normal muscle[59] and it seems reasonable to postulate that this Ca^{2+} release is exaggerated in MH muscle. Recent research using skinned muscle fibre preparations from human masseter muscle indicates why the increase in tone following succinylcholine is most pronounced in this one group of muscles.[4] It appears that the masseter muscle contains a remarkably high proportion of type I fibres. The myofilaments of type I fibres are more sensitive to Ca^{2+} than those of type II fibres[112] and so any increase in intracellular Ca^{2+} ion concentration in type I fibres is likely to produce greater contractile activity than a similar increase in type II fibres. This may be compounded by an increased sensitivity of the sarcoplasmic reticulum Ca^{2+} release channel in type I fibres to factors that promote Ca^{2+} release, such as caffeine and Ca^{2+} itself.[4]

There is debate about whether succinylcholine alone produces the metabolic features of MH, but a recent well documented case demonstrates that this indeed does occur, albeit to a mild degree.[80] What is clear is that administration of succinylcholine following an inhalation induction of anaesthesia with a volatile anaesthetic drug is more likely to produce masseter spasm and to produce the most rapid development of a severe MH reaction.

Non-depolarizing neuromuscular blocking drugs are now generally accepted to be safe in MH. Some authorities recommend that tubocurarine be avoided in MH-susceptible individuals as this drug has been shown to cause a small depolarization of denervated skeletal muscle[45] and was implicated in an early case report.[12] With the decreasing clinical usage of tubocurarine, this point is, however, becoming of only historical interest.

Phenothiazines

Phenothiazines were implicated in some of the early reports of MH as they were one of several groups of drugs given to patients who developed suspected MH reactions. There was even a case report of an MH reaction after premedication with trimeprazine before general anaesthesia was instated.[100] It was because of these reports that phenothiazines and related drugs, including other major and minor tranquillizers, entered the literature as possible triggers of MH. Now that they have entered the literature, it has proved difficult to discount completely the possibility that these drugs do indeed trigger MH. It is most likely that they are not triggers of MH. Several arguments can be put forward to support this view. In many of the case reports implicating phenothiazines, potent inhalational anaesthetics were also used. There is also some doubt as to whether some of the reactions reported as MH were true MH responses; this includes the report of MH following premedication with trimeprazines.[100] No case of MH following phenothiazine use alone has been verified by contracture test diagnosis on the patient concerned. Phenothiazines have anticholinergic actions, including antidiaphoresis, which can—especially in children—limit heat loss to such an extent that the body temperature will increase. MH has been confused with neuroleptic malignant syndrome,[48] which has similar clinical features to MH (pyrexia, muscle rigidity and rhabdomyolysis) but develops over 24–72 h and results from the central antidopaminergic effects of major tranquillizer drugs.[3,5]

In vitro studies of the effect of phenothiazines demonstrate that they can induce muscle contracture, but this occurs at concentrations far beyond those achieved with therapeutic concentrations of the drug.[2] These studies, however, have not dispelled completely the concern over phenothiazines and related drugs in MH. Perhaps the most reassuring factor in the use of phenothiazines and other major and minor tranquillizers in MH-susceptible patients is that there have been no reports of MH associated with usage of the drugs outside anaesthetic practice. Given the widespread use of these drugs and an incidence of MH of one in 8500 in the population, it would be anticipated that several cases should have occurred if the drugs were true triggers of MH.

Intravenous anaesthetic drugs

There is now vast experience of the safe use of the more commonly used intravenous anaesthetic drugs in patients who are known to be susceptible to MH. This includes the three most commonly used agents in current clinical practice, thiopental, etomidate and propofol. There was concern over the capacity of ketamine to induce a MH response, but the tachycardia and hypertension observed in MH-susceptible pigs and humans is probably a result of the usual sympathomimetic response to ketamine. Indeed, there is evidence that ketamine will in fact reduce Ca^{2+} release in skeletal muscle.[94]

Local anaesthetics

Ester local anaesthetics, specifically procaine, formed part of the limited treatment regimen for an MH reaction before the introduction of dantrolene,[51,58] although its value was questioned.[18,98] That ester local anaesthetics have some efficacy in MH is a result of their ability to reduce calcium release from the sarcoplasmic reticulum of skeletal muscle.[128] It is probably also for this reason that direct injection of ester local anaesthetics into myotonic muscles can relieve the myotonia.[61] On the other hand, early experiments with lidocaine, an amide local anaesthetic drug, showed that it induced *in vitro* contracture in skeletal muscle.[9] This led to amide local anaesthetics becoming contraindicated in MH-susceptible individuals as potential triggering drugs. It was soon realized, however, that the initial *in vitro* studies with lidocaine used inappropriately high concentrations of the drug, which could induce *in vitro* contracture even in normal muscle. Subsequent studies in pigs susceptible to porcine stress syndrome demonstrated that lidocaine did not trigger a reaction.[127] More recent experiments with mepivacaine indicate that MH muscle is perhaps more sensitive to the contracture-inducing effects of amide local anaesthetics (E. Hartung, personal communication), but again the concentration of mepivacaine

required to produce contracture in MH muscle is still far in excess of that achieved systemically in normal clinical practice.

We now use femoral nerve block with amide local anaesthetic to provide analgesia for the diagnostic muscle biopsy of patients potentially susceptible to MH as our standard practice in the UK MH Investigation Unit in Leeds. Initially, lidocaine and bupivacaine were used. Lidocaine has since been replaced by prilocaine, which has a higher therapeutic index for neurological and cardiac complications. We have used prilocaine 5 mg kg^{-1} in >1000 patients who proved to be susceptible to MH. This dose of prilocaine is sufficient to cause clinically apparent methaemaglobinaemia in some patients[8] and, in one case, inadvertent intravascular injection led to fitting, but there has been no indication of triggering of MH in any patient.

Other drugs

As alluded to earlier, anticholinergic drugs can exacerbate pyrexia because of their anti-diaphoretic action. This may make treatment of an MH reaction triggered by, for example, potent inhalational anaesthetics, more difficult but should not preclude their use in MH-susceptible individuals.[102] There has also been uncertainty over the use of catecholamines and other sympathomimetic drugs which has arisen in the context of conflicting evidence from studies in pigs with porcine stress syndrome (porcine MH).[26 46 47 85 88] As porcine stress syndrome is triggered by stress, whereas human MH is not, it would seem reasonable to assume that MH-susceptible humans would be less likely to respond abnormally to catecholamines than are pigs. The lack of response in pigs with porcine stress syndrome to intravenous infusion of norepinephrine[46] is reassuring from the human perspective.

One group of drugs that could trigger MH are those having similar structure or actions to caffeine, one of the compounds used for *in vitro* diagnosis of the condition.[35] Theophylline and aminophylline do indeed cause *in vitro* contracture in muscle and, at lower concentration, in muscle from MH individuals (unpublished observations), but these concentrations are several times greater than those achieved with therapeutic use. Caffeine and other methylxanthines are non-specific phosphodiesterase inhibitors and it is possible that this effect contributes to the sensitivity of MH muscle to caffeine. Specific inhibitors of type III phosphodiesterase have gained a place in the management of heart failure and as inotropes in cardiac surgery. One of these, enoximone, has been evaluated for its effect on isolated human muscle from normal and MH-susceptible individuals.[34] As with the methylxanthines, enoximone produced a concentration-dependent contracture, with the dose–response curve shifted to the left in muscle from MH-susceptible individuals. The minimum concentration for contracture development was 100 times greater than has been measured with clinical use of enoximone.[34]

Advances in the laboratory diagnosis of MH

The two widely used protocols for the diagnosis of MH susceptibility using IVCTs with living skeletal muscle[35 36 75] both use separate exposure to halothane and to caffeine. The protocol of the North American MH Group also allowed for a combined caffeine–halothane test,[75] but this has been largely abandoned because of a high incidence of false-positive results.[32 77] The separate-exposure halothane and caffeine tests also have their imperfections. Results of IVCT that have been done on patients with a low risk of MH ('controls') suggest a false-positive rate of approximately 6% using the European protocol[103] and 9% using the North American protocol.[77] In results from the Leeds MH Investigation Unit, 14% of patients with an abnormal response to halothane reacted normally to caffeine, indicating that the caffeine test lacks total sensitivity. A report of complete failure of the European MH Group protocol to detect cases of MH susceptibility,[70] however, has not stood up to close scrutiny, both in terms of strict application of the test protocol and the clinical diagnoses of MH.

The halothane and caffeine IVCTs have proved satisfactory for clinical diagnostic purposes over the past 30 years. The lack of specificity of both tests and the lack of sensitivity of the caffeine test do, however, pose problems for genetic studies of MH where accurate phenotyping is essential. One approach to improving accuracy is to assess other drugs that induce *in vitro* muscle contracture for their ability to distinguish MH-susceptible from normal muscle. Two drugs, ryanodine and chlorocresol, have shown real potential.

Ryanodine

Ryanodine, formerly used as an insecticide, was shown in the late 1950s to cause time- and dose-dependent contractures of normal rabbit and rat skeletal muscle.[114] It was subsequently suggested that ryanodine could be used to produce an animal model for MH.[16 38] Unfortunately, this idea was not developed further and ryanodine was neglected by MH researchers until the late 1980s when it was described as having great affinity for the sarcoplasmic reticulum calcium release channel.[91] Indeed, ryanodine was so important in the purification and characterization of this calcium release channel that it became known as the ryanodine receptor protein.[15] It was then not long before the ryanodine receptor protein was suggested to be the site of the MH defect because [^{3}H]ryanodine was shown to have greater affinity for the protein from pigs with porcine stress syndrome than for that from normal pigs.[97]

With the publication of these studies it seemed clear that ryanodine was worth investigating for its effect on human MH muscle. Following initial dose-finding studies using rat diaphragm and human tissue, a protocol was devised for a pilot study to compare responses to ryanodine of muscle from MH-susceptible and normal individuals.[63] The results

of the pilot study suggested that the ryanodine contracture test could be specific for MH. It was also interesting that muscle from two patients with equivocal responses to the standard halothane and caffeine tests (MHE) behaved in a similar way to that of the susceptible patients.[63] A larger series of patients was included in a further study using a refined ryanodine application protocol.[64] The results again gave credence to the possibility that ryanodine contracture testing was able to distinguish MH-susceptible from normal patients. Furthermore, they appeared to identify two populations within the MHE group, which suggested the test had the potential to distinguish positive and negative patients within this group—a great asset for future genetic analyses.[64]

Similar results were reported subsequently by other groups,[56 82 124 125] but each group was using its own protocol. Differences in the protocols included the purity of the ryanodine used, the concentrations of ryanodine applied, whether a bolus or incremental addition of ryanodine was used and the criteria for determining the end-points of the test. Although most studies reported good discrimination of MH-susceptible and normal patients, it was very difficult to compare the results between groups.

At its meeting in Padua in 1993, the European MH Group agreed the necessity for a common protocol for the ryanodine contracture test if the compound was to be evaluated as an additional test drug. There was a need to confirm that ryanodine could distinguish between MH-susceptible and normal in a large series of patients and that the test was reproducible between laboratories. The results of the multicentre evaluation of this protocol were published in 1998.[67] The study enabled the determination of cut-off values that can be used to categorize a ryanodine contracture test response as MH-susceptible or normal with varying degrees of probability.

There were statistical differences found in ryanodine responses between several centres, but in the majority of cases these were not large enough to compromise the use of common parameters for discriminating between 'normal' and 'abnormal' ryanodine contracture responses.[67] An analogous situation exists with the diagnostic halothane and caffeine tests. In these tests, common threshold concentrations of halothane and caffeine are successfully used by all centres, but the size of contracture developed at threshold concentrations does vary between centres. The ryanodine contracture test results of one centre, however, were markedly different from those of the other centres. These differences were so marked that, at the time of publication of the paper,[67] they raised a great deal of uncertainty about the use of the ryanodine contracture test. It has transpired subsequently that the centre producing the disparate results had been stimulating the muscle at 10 times the agreed rate. The study of Sudo and Nelson[118] demonstrated how this can influence responses to ryanodine. The North American MH Group have subsequently adopted the European MH Group protocol for the ryanodine contracture test for the use of their members who wish to use a ryanodine test.

Future use of the ryanodine contracture test

We have examined the predictive probability of the various contracture tests in determining MH susceptibility.[66] Interestingly, the rank order of correlation with MH diagnosis of the four tests studied was ryanodine > dynamic halothane > static halothane > caffeine test. Although we would not advise abandoning tests with halothane and caffeine, our results suggest that an additional ryanodine contracture test is of value. Furthermore, in the same paper,[66] we demonstrated how ryanodine contracture test results could be combined with results of the other tests in a mathematical model to produce a sensitive and specific estimate of the probability of an individual being truly MH susceptible. This has potential application in producing a quantitative phenotype for genetic analyses which would enable the inclusion of MHE patients in such analyses. A further use for the ryanodine contracture test is in comparison of results produced by the two major protocols for IVCT, those of the North American and European MH Groups.[40]

Chlorocresol

4-Chloro-*m*-cresol (4-CmC) is used as a preservative in some pharmaceutical preparations of, for example, insulin, heparin and succinylcholine. It affects the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum and causes time- and dose-dependent muscle contracture.[119] Early studies, each using a different protocol, have shown promise similar to ryanodine.[44 104 119 126] Multicentre evaluation of a common protocol is in progress.

Molecular genetics of MH

Early genetic linkage studies found linkage to chromosome 19q12.1-13.2,[90 95] the locus of the ryanodine receptor gene (*RYR1*), the gene encoding the skeletal muscle sarcoplasmic reticulum Ca^{2+} release channel.[89] As further families have been investigated, though, it has become apparent that the molecular genetics of MH are more complex.[65] To date, fewer than 60 families worldwide have proved large enough to provide informative genetic data. These data have demonstrated genetic heterogeneity[23 83] with the results from 30–80% of families consistent with linkage to *RYR1*. In families where linkage data have excluded the *RYR1* locus, further linkage analysis to loci of physiologically plausible candidate genes, such as the subunits of the dihydropyridine (DHPR) membrane Ca^{2+} channel, have been carried out. No linkage has been verified (but see references 72 and 84) to the β or γ subunits,[68 116] but one German pedigree is significantly linked to the region of the gene encoding the $\alpha 2/\delta$ subunit; this locus is found on chromosome 7q.[69] A genomic search identified linkage in a French family to the gene encoding the $\alpha 1$ DHPR subunit on chromosome 1q[110]

and a mutation in this gene was subsequently found in MH-susceptible members of this family.[99]

Genomic searches have identified two further loci, on chromosomes 3q[117] and 5p,[110] to which MH susceptibility was linked in one family in each case. Subsequent studies using other families have failed to find other pedigrees in which MH susceptibility is linked to these loci.

Developments have also taken place in the search for mutations of *RYR1* that may be causative of MH. The first human mutation was found[43] following the discovery that an arginine to cysteine substitution in amino acid position 614 of *RYR1* in pigs was associated with porcine stress syndrome.[42] The mutation underlying this substitution, C1840T, in humans has been found in 14 out of 333 susceptible individuals from separate MH pedigrees in Europe (European MH Group, unpublished data). Fifteen further mutations have been found in one or more MH families.[74 92 93 106–108] Of these mutations, the commonest is the G1021A mutation, which has been found in 19 out of 298 susceptible individuals screened in Europe (European MH Group, unpublished data). When the extended pedigrees of these families are examined, however, there is discordance between inheritance of the mutation and MH susceptibility in five families (European MH Group, unpublished data). There is a similar situation with the C1840T mutation[24 37] where discordance is present in four out of 14 families investigated (European MH Group, unpublished data). Our study of a large UK pedigree[1] in which the G1021A mutation is found in only seven of 12 MH-susceptible individuals, points to a second underlying genetic abnormality causing MH in this family. While it is difficult to envisage that the mutations so far described in *RYR1* do not play a role in MH, and while this is supported by functional expression studies,[120 121] there is such a high rate of discordance of the two more common mutations that these may well be only modifying factors in expression of the disorder.

The complexity of the molecular genetics of MH described above precludes DNA-based diagnosis at present, especially when one considers the possibility of one gene defect being associated with susceptibility in only a proportion of individuals and another, as yet unidentified, defect being causative of the condition. This is complicated further by the well-established presence of genetic heterogeneity. The first step in a DNA-based diagnosis, therefore, will rely on initial identification of the abnormality, or abnormalities, causing MH in individual pedigrees. It is likely that the first reliable DNA-based diagnoses will be carried out in individuals from families that have been extensively investigated by both IVCT phenotyping and linkage analysis followed by mutation screening.

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